

WHAT IS CLAIMED IS:

1. A gene which encodes reverse transcriptase having DNA polymerase activity and substantially no RNase H activity.

2. The gene of claim 1, wherein said gene is derived from an organism selected from the group consisting of Moloney murine leukemia virus (M-MLV), human T-cell leukemia virus type I (HTLV-I), bovine leukemia virus (BLV), Rous sarcoma virus (RSV), human immunodeficiency virus (HIV), yeast, Neurospora, Drosophila, primates and rodents.

3. The gene of claim 1, wherein said microorganism is M-MLV, comprising the following DNA sequence:

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1078
ATG ACC CTA AAT ATA GAA GAT GAG CAT CCG CTA CAT GAG ACC TCA AAA GAG CCA GAT GGT
1138
TCT CTA GGG TCC ACA TGG CIG TCT GAT TTT OCT CAG GGC TGG GCG GAA ACC GGG GGC ATG
1198
GGA CIG GCA GTT CCG CAA GCT OCT CIG ATC ATA OCT CIG AAA GCA ACC TCT ACC CCG GIG
1258
TCC ATA AAA CAA TAC CCC ATG TCA CAA GAA GGC AGA CIG GGG ATC AAG CCC CAC ATA CAG
1318
AGA CIG TTG GAC CAG GGA ATA CIG GTA CCC TGC CAG TCC CCC TGG AAC AGC CCC CIG CTA
1378
CCC GGT AAG AAA CCA GGG ACT AAT GAT TAT AGG CCT GTC CAG GAT CIG AGA GAA GTC AAC
1438
AAG CCG GTG GAA GAC ATC CAC CCC ACC GIG CCC AAC OCT TAC AAC CTC TTG AGC GGG CTC
1498
CCA CCG TCC CAC CAG TGG TAC ACT GIG CTT GAT TTA AAG GAT GGC TTT TTC TGC CIG AGA
1558
CTC CAC CCC ACC AGT CAG OCT CTC TTC GGC TTT GAG TGG AGA GAT CCA GAG ATG GCA ATC
1618
TCA GCA CAA TTG ACC TGG ACC AGA CTC CCA CAG GGT TTC AAA AAC AGT CCC ACC CIG TTT
1678
GAT GAG GCA CIG CAC AGA GAC CTA GCA GAC TTC CCG ATC CAG CAC CCA GAC TTG ATC CIG
1738
CTA CAG TAC GIG GAT GAC TTA CIG CIG GGC GGC ACT TCT CAG CTA GAC TGC CAA CAA GGT
1798
ACT CCG GGC CIG TTA CAA ACC CTA GGG AAC CTC GGG TAT CCG GGC TCG GGC AAG AAA GGC
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1858
 CAA ATT TGC CAG AAA CAG GTC AAG TAT CIG GGG TAT CTT CTA AAA GAG GGT CAG AGA TGG
 1918
 CTG ACT GAG GGC AGA AAA GAG ACT GIG AIG GGG CAG OCT ACT CCG AAG ACC OCT GSA CAA
 1978
 CTA AGG GAG TTC CTA GGG AGG GCA GGC TTC TGT GGC CTC TGG ATC OCT GGG TTT GCA CAA
 2038
 AIG GCA GGC CCC TTG TAC OCT CTC ACC AAA AGG GGG ACT CIG TTT AAT TGG GGC CCA GAC
 2098
 CAA CAA AAG GGC TAT CAA GAA ATC AAG CAA GCT CTT CTA ACT GGC CCA GGC CIG GGG TTG
 2158
 CCA GAT TTG ACT AAG CCC TTT GAA CTC TTT GTC GAC GAG AAG CAG GGC TAC GGC AAA GGT
 2218
 GTC CTA AAG CAA AAA CIG GCA OCT TGG GGT CCG CCG GIG GGC TAC CIG TOC AAA AAG CTA
 2278
 GAC CCA GTA GCA GCT GGG TGG CCC OCT TGC CTA GGG AIG GTA GCA GGC AAT GGC GTA CIG
 2338
 ACA AAG GAT GCA GGC AAG CTA ACC AIG GCA CAG CCA CTA GTC AAT CIG GGC CCC CAT GCA
 2398
 GTA GAG GCA CTA GTC AAA CAA CCC CCC GAC GGC TGG CTT TOC AAC GGC CCG AIG CAT CAC
 2458
 TAT CAG GGC TTG CTT TTG GAC AGG GAC GGG GTC CAG TTC GSA CCG GIG GTA GGC CIG AAC
 CCG GCT AAG CIG CTC CCA CIG OCT GAG GAA GGG CIG CAA CAC AAC TGC CTT GAT

or the degenerate variants thereof.

4. The gene of claim 1, wherein said micro-organism is M-MLV, comprising the following DNA sequence:

1078
 AIG ACC CTA AAT ATA GAA GAT GAG CAT CCG CTA CAT GAG ACC TCA AAA GAG CCA GAT GAT
 1138
 TCT CTA GGG TOC ACA TGG CIG TCT GAT TTT OCT CAG GGC TGG GGG GAA ACC GGG GGC AIG
 1198
 GGA CIG GCA GAT GGC CAA GCT OCT CIG ATC ATA OCT CIG AAA GCA ACC TCT ACC CCC GIG
 1258
 TOC ATA AAA CAA TAC CCC AIG TCA CAA GAA GGC AGA CIG GGG ATC AAG CCC CAC ATA CAG
 1318
 AGA CIG TTG GAC CAG GSA ATA CIG GTA CCC TGC CAG TOC CCC TGG AAC AGG CCC CIG CTA
 1378
 CCC GAT AAG AAA CCA GGG ACT AAT GAT TAT AGG OCT GTC CAG GAT CIG AGA GAA GTC AAC
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1438
AAG CGG GIG GAA GAC AIC CAC CCC AOC GIG CCC AAC OCT TAC AAC CIG TTG AGC GGG CIG

1498
CCA CCG TOC CAC CAG TGG TAC ACT GIG CTT GAT TTA AAG GAT GOC TIT TTC TGC CIG AGA

1558
CIC CAC CCC AOC AGT CAG OCT CIG TTC GOC TIT GAG TGG AGA GAT CCA GAG AIG GGA AIC

1618
TCA GGA CAA TTG AOC TGG AOC AGA CIG CCA CAG GGT TTC AAA AAC AGT CCC AOC CIG TIT

1678-
GAT GAG GCA CIG CAC AGA GAC CTA GCA GAC TTC CGG AIC CAG CAC CCA GAC TTG AIC CIG

1738
CFA CAG TAC GIG GAT GAC TTA CIG CIG GOC GOC ACT TCT GAG CTA GAC TGC CAA CAA GGT

1798
ACT CGG GOC CIG TTA CAA AOC CTA GGG AAC CIG GGG TAT CGG GOC TGG GOC AAG AAA GOC

1858
CAA AIT TGC CAG AAA CAG GIC AAG TAT CIG GGG TAT CTT CTA AAA GAG GGT CAG AGA TGG

1918
CIG ACT CAG GOC AGA AAA CAG ACT GIG AIG GGG CAG OCT ACT CCG AAG AOC OCT CCA CAA

1978
CFA AGG GAG TTC CTA GGG AOG GCA GGC TTC TGT CSC CIG TGG AIC OCT GGG TIT GCA GAA

2038
AIG GCA GOC CCC TTG TAC OCT CIG AOC AAA ACG GGG ACT CIG TIT AAT TGG GGC CCA GAC

2098
CAA CAA AAG GOC TAT CAA GAA AIC AAG CAA GCT CTT CTA ACT GOC CCA GOC CIG GGG TTG

2158
CCA GAT TTG ACT AAG CCC TIT GAA CIG TIT GIC GAC GAG AAG CAG GGC TAC GOC AAA GGT

2218
GIC CTA ACG CAA AAA CIG GGA OCT TGG GGT CCG CCG GIG GOC TAC CIG TOC AAA AAG CTA

2278
GAC CCA GTA GCA GCT GGG TGG CCC OCT TGC CTA CCG AIG GFA GCA GOC AIT GOC GFA CIG

2338
ACA AAG GAT GCA GGC AAG CTA AOC AIG GGA CAG CCA CTA GIC AIT CIG GOC CCC CAT GCA

2398
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GTA GAG GCA CTA GTC AAA CAA CCC CCC GAC CGC TGG CTT TCC AAC GGC CGG ATG ACT CAC
2458
TAT CAG GGC TGG CTT TTG GAC ACG GAC CGG GTC CAG TTC GGA CCG GTG GGA GGC CTG AAC
2518
CCG GGT ACG CTG CTC CCA CTG CTT GAG GAA GGG CTG CAA CAC AAC TGC CTT GAT AAT TCC
2530
CGC TTA ATT AAT

or the degenerate variants thereof.

5. A gene which encodes a fusion protein which comprises reverse transcriptase having DNA polymerase activity and substantially no RNase H activity and a second protein comprising a hydrophobic leader peptide or a stabilizing peptide.

6. A vector containing the gene of claim 1 or 5.

7. The vector of claim 6 designated pRTdEcoRV-C which has been deposited at the American Type Culture Collection, Rockville Maryland under terms of the Budapest Treaty and given accession number 67555.

8. A host transformed with the vector of claim 6.

9. A polypeptide having an amino acid sequence encoded by the cloned gene of claim 1 or 5.

10. The polypeptide of claim 9 comprising the following amino acid sequence:

MET Thr Leu Asn Ile Glu Asp Glu His Arg Leu His Glu Thr Ser Lys Glu Pro Asp Val
 Ser Leu Gly Ser Thr Trp Leu Ser Asp Phe Pro Gln Ala Trp Ala Glu Thr Gly Gly MET
 Gly Leu Ala Val Arg Gln Ala Pro Leu Ile Ile Pro Leu Lys Ala Thr Ser Thr Pro Val
 Ser Ile Lys Gln Tyr Pro MET ser Gln Glu Ala Arg Leu Gly Ile Lys Pro His Ile Gln
 Arg Leu Leu Asp Gln Gly Ile Leu Val Pro Cys Gln Ser Pro Trp Asn Thr Pro Leu Leu
 Pro Val Lys Lys Pro Gly Thr Asn Asp Tyr Arg Pro Val Gln Asp Leu Arg Glu Val Asn
 Lys Arg Val Glu Asp Ile His Pro Thr Val Pro Asn Pro Tyr Asn Leu Leu Ser Gly Leu
 Pro Pro Ser His Gln Trp Tyr Thr Val Leu Asp Leu Lys Asp Ala Phe Phe Cys Leu Arg
 Leu His Pro Thr Ser Gln Pro Leu Phe Ala Phe Glu Trp Arg Asp Pro Glu MET Gly Ile
 Ser Gly Gln Leu Thr Trp Thr Arg Leu Pro Gln Gly Phe Lys Asn Ser Pro Thr Leu Phe
 Asp Glu Ala Leu His Arg Asp Leu Ala Asp Phe Arg Ile Gln His Pro Asp Leu Ile Leu
 Leu Gln Tyr Val Asp Asp Leu Leu Leu Ala Ala Thr Ser Glu Leu Asp Cys Gln Gln Gly
 Thr Arg Ala Leu Leu Gln Thr Leu Gly Asn Leu Gly Tyr Arg Ala Ser Ala Lys Lys Ala
 Gln Ile Cys Gln Lys Gln Val Lys Tyr Leu Gly Tyr Leu Leu Lys Glu Gly Gln Arg Trp
 Leu Thr Glu Ala Arg Lys Glu Thr Val MET Gly Gln Pro Thr Pro Lys Thr Pro Arg Gln
 Leu Arg Glu Phe Leu Gly Thr Ala Gly Phe Cys Arg Leu Trp Ile Pro Gly Phe Ala Glu
 MET Ala Ala Pro Leu Tyr Pro Leu Thr Lys Thr Gly Thr Leu Phe Asn Trp Gly Pro Asp
 Gln Gln Lys Ala Tyr Gln Glu Ile Lys Gln Ala Leu Leu Thr Ala Pro Ala Leu Gly Leu
 Pro Asp Leu Thr Lys Pro Phe Glu Leu Phe Val Asp Glu Lys Gln Gly Tyr Ala Lys Gly
 Val Leu Thr Gln Lys Leu Gly Pro Trp Arg Arg Pro Val Ala Tyr Leu Ser Lys Lys Leu
 Asp Pro Val Ala Ala Gly Trp Pro Pro Cys Leu Arg MET Val Ala Ala Ile Ala Val Leu
 Thr Lys Asp Ala Gly Lys Leu Thr MET Gly Gln Pro Leu Val Ile Leu Ala Pro His Ala
 Val Glu Ala Leu Val Lys Gln Pro Pro Asp Arg Trp Leu Ser Asn Ala Arg MET Thr His

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Tyr Gln Ala Leu Leu Leu Asp Thr Asp Arg Val Gln Phe Gly Pro Val Val Ala Leu Asn
Pro Ala Thr Leu Leu Pro Leu Pro Glu Glu Gly Leu Gln His Asn Cys Leu Asp.

11. The polypeptide of claim 9 comprising the following amino acid sequence:

MET Thr Leu Asn Ile Glu Asp Glu His Arg Leu His Glu Thr Ser Lys Glu Pro Asp Val
Ser Leu Gly Ser Thr Trp Leu Ser Asp Phe Pro Gln Ala Trp Ala Glu Thr Gly Gly MET
Gly Leu Ala Val Arg Gln Ala Pro Leu Ile Ile Pro Leu Lys Ala Thr Ser Thr Pro Val
Ser Ile Lys Gln Tyr Pro MET ser Gln Glu Ala Arg Leu Gly Ile Lys Pro His Ile Gln
Arg Leu Leu Asp Gln Gly Ile Leu Val Pro Cys Gln Ser Pro Trp Asn Thr Pro Leu Leu
Pro Val Lys Lys Pro Gly Thr Asn Asp Tyr Arg Pro Val Gln Asp Leu Arg Glu Val Asn
Lys Arg Val Glu Asp Ile His Pro Thr Val Pro Asn Pro Tyr Asn Leu Leu Ser Gly Leu
Pro Pro Ser His Gln Trp Tyr Thr Val Leu Asp Leu Lys Asp Ala Phe Phe Cys Leu Arg
Leu His Pro Thr Ser Gln Pro Leu Phe Ala Phe Glu Trp Arg Asp Pro Glu MET Gly Ile
Ser Gly Gln Leu Thr Trp Thr Arg Leu Pro Gln Gly Phe Lys Asn Ser Pro Thr Leu Phe
Asp Glu Ala Leu His Arg Asp Leu Ala Asp Phe Arg Ile Gln His Pro Asp Leu Ile Leu
Leu Gln Tyr Val Asp Asp Leu Leu Leu Ala Ala Thr Ser Glu Leu Asp Cys Gln Gln Gly
Thr Arg Ala Leu Leu Gln Thr Leu Gly Asn Leu Gly Tyr Arg Ala Ser Ala Lys Lys Ala
Gln Ile Cys Gln Lys Gln Val Lys Tyr Leu Gly Tyr Leu Leu Lys Glu Gly Gln Arg Trp
Leu Thr Glu Ala Arg Lys Glu Thr Val MET Gly Gln Pro Thr Pro Lys Thr Pro Arg Gln
Leu Arg Glu Phe Leu Gly Thr Ala Gly Phe Cys Arg Leu Trp Ile Pro Gly Phe Ala Glu
MET Ala Ala Pro Leu Tyr Pro Leu Thr Lys Thr Gly Thr Leu Phe Asn Trp Gly Pro Asp
Gln Gln Lys Ala Tyr Gln Glu Ile Lys Gln Ala Leu Leu Thr Ala Pro Ala Leu Gly Leu
Pro Asp Leu Thr Lys Pro Phe Glu Leu Phe Val Asp Glu Lys Gln Gly Tyr Ala Lys Gly
Val Leu Thr Gln Lys Leu Gly Pro Trp Arg Arg Pro Val Ala Tyr Leu Ser Lys Lys Leu

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Asp Pro Val Ala Ala Gly Trp Pro Pro Cys Leu Arg MET Val Ala Ala Ile Ala Val Leu
Thr Lys Asp Ala Gly Lys Leu Thr MET Gly Gln Pro Leu Val Ile Leu Ala Pro His Ala
Val Glu Ala Leu Val Lys Gln Pro Pro Asp Arg Trp Leu Ser Asn Ala Arg MET Thr His
Tyr Gln Ala Leu Leu Leu Asp Thr Asp Arg Val Gln Phe Gly Pro Val Val Ala Leu Asn
Pro Ala Thr Leu Leu Pro Leu Pro Glu Glu Gly Leu Gln His Asn Cys Leu Asp Asn Ser
Arg Leu Ile Asn.

12. A method of producing reverse transcriptase having DNA polymerase activity and substantially no RNase H activity comprising culturing the transformed host of claim 8 under conditions which produce reverse transcriptase, and isolating the reverse transcriptase so produced.

13. A method of preparing cDNA from mRNA, comprising

(a) contacting mRNA with an oligo(dT) primer or other complementary primer to form a hybrid, and

(b) contacting said hybrid formed in step (a) with reverse transcriptase, having DNA polymerase and substantially no RNase activity, and the nucleoside triphosphates to give a cDNA-RNA hybrid.

14. The method of claim 13, further comprising treating the cDNA-RNA with alkali or RNase H to selectively hydrolyze said RNA to give a cDNA.

15. The method of claim 13, further comprising treating said cDNA with DNA polymerase to give second-strand cDNA.

16. A kit for the preparation of cDNA from mRNA, comprising a carrier means being compartmentalized to receive in close confinement therein, one or more containers wherein

(a) a first container contains reverse transcriptase having DNA polymerase activity and substantially no RNase H activity;

(b) a second container contains the nucleoside triphosphates, and

(c) a third container contains oligo(dT) primer.

17. The kit of claim 16, further comprising:

(d) a fourth container containing control RNA.

18. The kit of claim 16, wherein said second container further contains a buffer.

19. The kit of claim 16, wherein said reverse transcriptase is present at a concentration of 200 $\mu\text{g}/\mu\text{l}$ to 400 $\mu\text{g}/\mu\text{l}$.

20. The kit of claim 16, wherein said oligo (dT) primer is present at a concentration of 5 $\mu\text{g}/\text{ml}$ to 20 $\mu\text{g}/\text{ml}$.

21. The kit of claim 18, wherein said buffer comprises Tris-HCl (pH 7.5 to 8.3), KCl, MgCl_2 , and dithiothreitol.

22. The kit of claim 16, wherein said nucleoside triphosphates are present at a concentration of 0.5 mM to 2 mM.

23. The kit of claim 17, wherein said control RNA is present at a concentration of 10 $\mu\text{g/ml}$ to 20 $\mu\text{g/ml}$.

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